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PENSOFT

First record of the genus *Micromeriella* Betrem, 1972 (Hymenoptera, Scoliidae) from the Maltese Islands, central Mediterranean

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Abstract

Here we report the first distribution record of the genus *Micromeriella* Betrem, 1972 (Hymenoptera, Scoliidae) from the archipelago of Malta, based on two specimens of *Micromeriella aurolea* (Klug, 1832) collected from the island of Gozo. Although this species is relatively well distributed in the Afrotropical and Palaearctic regions, we extend its distribution to the central Mediterranean islands. Molecular barcoding using the cytochrome c oxidase subunit I (COI) gene was conducted and compared with the known COI sequences of the genus to confirm the identification of the species.

Keywords

Biomonitoring, DNA barcoding, Malta, new record, scoliid wasp

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Introduction

The family Scoliidae comprises 560 valid species of wasps belonging to 43 genera (Osten 2005a), commonly known as hairy wasps. Most of these medium-sized to large wasps exhibit sexual dimorphism, with males usually having longer antennae and a more slender elongated abdomen than their female counterparts (Osten 2000; Osten 2005a; Kim 2009; Nidup 2017). Scoliid larvae are parasitoids of beetle larvae and have co-evolved with their hosts, namely those of the family Scarabaeidae, while the adult wasps feed on pollen and nectar (Clausen et al. 1932; Gupta and Jonathan 2003; Gadallah 2004; Osten 2005a; Samin et al. 2014). Species of scoliid wasps currently known in Malta include: *Campsomeriella thoracica thoracica* (Fabricius, 1787); *Dasyscolia ciliata ciliata* (Fabricius, 1787); *Megascolia bidens* (Fabricius, 1775); *Megascolia flavifrons* (Fabricius, 1775); *Scolia hirta unifasciata* (Cyirillo, 1787); and *Scolia sexmaculata sexmaculata* (Müller, 1766) (Osten 1994; Sultana and Falzon 2002; Samin et al. 2014; Demetriou et al. 2021). Prior to this study, there were no reports on the occurrence of *Micromeriella* Betrem, 1972 species in Malta.

The occurrence of *Micromeriella* specimens on the island of Gozo (Maltese archipelago) deserved further investigation, given that its recent presence may be indicative of changes towards drier and warmer environmental conditions while its larvae are parasitoid of beetles. Here we report the first record of *Micromeriella* from Malta, using both morphological and genetic characters

to identify the specimens collected down to the species level. The latter was necessary given the great similarity between *Micromeriella aureola* (Klug, 1832) and *Micromeriella hyalina* (Klug, 1832), as highlighted by Osten (2000).

Methods

Specimens included in our study were collected from San Lawrenz, Gozo (Maltese Islands, central Mediterranean) (Fig. 1), and form part of an ongoing biodiversity research and monitoring project undertaken by the Conservation Biology Research Group, University of Malta (CBRG-UM). Upon collection, both specimens were photographed and were deposited at the wildlife species collection of the CBRG-UM, Msida, Malta as voucher specimens HymST224 and HymST225 (Fig. 2). Morphological identification of these specimens was based on Osten (2000), Gadallah (2004), and Schmid-Egger (2017).

Total genomic DNA was extracted from each specimen using GF-1 Tissue DNA Extraction Kit (Vivantis, Malaysia). The 658 bp barcode region of the mitochondrial cytochrome c oxidase subunit I (COI) was amplified using LCO1490 and HCO2198 primers (Folmer et al. 1994) following Mifsud et al. (2019). PCR products were subsequently purified and sequenced using both amplification primers via ABI3730XL sequencer. Quality check, editing, and assembly of complementary sequences were carried out using Geneious R10 (https://www.geneious.com; Kearse et al. 2012).

The final COI data were trimmed from primers leading to a 658 bp sequence for both specimens. The currently generated data were compared with the NCBI GenBank database via Blastn (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and to the BOLD Species Level Barcode Records Identification Engine (http://www.boldsystems.org; Ratnasingham and Hebert 2007) to identify the closest match and to which Barcode Index Number (BIN) the species belongs. The COI gene sequences from BOLD also allowed for phylogenetic analysis of these specimens, and comparative analyses of the currently analysed specimens to other scoliids. Sequences chosen were aligned using Geneious R10 (http://www.geneious.com, Kearse et al. 2012) and were trimmed to the size of the smallest homologous sequence. The genetic divergences between the COI sequences selected were calculated using the p-distance model via Arlequin v. 3.5 (Excoffier and Lischer 2010), and a neighbour-joining phylogenetic tree using *p*-distance (Collins et al. 2012; Srivathsan and Meier 2012) and 1500 bootstraps was constructed through MEGA v. 7 (Kumar et al. 2016). The genetic data generated during this study were deposited at GenBank under accession numbers MZ366358 and MZ366359.

Results

Family Scoliidae
Subfamily Scoliinae
Tribe Campsomerini
Genus *Micromeriella* Betrem, 1972

Figure 1. Map showing the sampling sites of *Micromeriella aureola*.

Figure 2. Habitus of *Micromeriella aureola* (scale bar: 5 mm).

***Micromeriella aureola* (Klug, 1832)**

New record. 2 ♀, MALTA • San Lawrenz, Gozo; 36°03′16″N, 014°11′42″E; 90 m a.s.l.; 3.X.2020; S. Tabone leg.; Mediterranean garrigue habitat; GenBank MZ366358 & MZ3663589; CBRG HymST224 & HymST225.

Diagnosis. The two specimens had a robust and densely light-haired body, with body lengths of 17.3 mm and 17.2 mm (Fig. 2). Both specimens had black short antennae (Fig. 2) indicating that both were females, given that like other scoliids this species exhibits sexual dimorphism where the males have much longer antennae that extend to more than half the body length (Osten 2000; Gadallah 2004; Osten 2005a; Pagliano 2017). The specimens were identified based on the colour characters described by Osten (2000) and Gadallah (2004). The wings were hyaline and had yellow veins, with the fore wing having two recurrent veins; the venation pattern matched that illustrated by Gadallah (2004) (Fig. 3A). Both specimens had a black head, with their occiput having long yellowish hairs (Fig. 3B, 3C). The clypeus (Fig. 3D) was black and covered with long whitish hairs, and its free margin was slightly lighter in colour and was longitudinally ridged as described by Gadallah (2004), which distinguished the analysed specimens from *M. hyalina*. The thorax was completely black and had a shiny mesoscutum that was deeply punctured laterally and was bare medially (Fig. 3C). The abdomen was characterized mainly by yellow hairs and had predominantly golden yellow terga, with the latter having black anterior margins that were most pronounced from T4 to T6, giving the abdomen an overall golden and black banding pattern. This banding pattern distinguished *Micromeriella* species from *C. thoracica*, given that females of the latter species are entirely black (Osten 2000; Gadallah 2004). In our specimens, the femora and tibiae were black, while the tarsi were brown-orange, and all legs were covered with long, light-coloured hairs. Along the length of the middle and hind tibiae were longitudinal rows of curved, transparent spines arising from serrations (Fig. 4A). The outer spur on the hind tibia was spatulate and apically enlarged (Fig. 4A, 4B), a feature that is characteristic to *M. aureola* and can be used to distinguish it from its closely related conspecific *M. hyalina* given that the corresponding spur in the *M. hyalina* is narrow and pointed (Gadallah 2004; Schmid-Egger 2017).

Remarks. At subspecies level, our specimens were neither *M. aureola aureola* (Klug, 1832), as in this subspecies the abdomen and the tibia on the middle and hind legs are almost completely yellow, nor *M. aureola elegans* (Brulle, 1840) as in this subspecies the orange bands on the tergites are constricted or interrupted in the middle, while the tibia on the middle and hind leg are also orange-red (Osten 2000). The elimination of the aforementioned subspecies and the presence of a lateral brown spot on the second tergite (Fig. 4A) are indicative of *M. aureola dimidiata* (Lepeletier, 1845) from the Mediterranean region, a subspecies associated with Morocco and Tunisia, as indicated by Osten (2000).

Distribution. Afrotropical regions and Palaearctic regions ranging from the Canary Islands to northern and north-eastern Africa to Iran (Osten 2000; Schmid-Egger 2017), including the Canary Islands, Morocco to Sudan (Osten 2000), Djibouti (Madl 2018); Saudi Arabia (Gadallah 2004); United Arab Emirates (Schmid-Egger 2017); Oman (Osten 2005b), and Iran (Osten et al. 2003; Samin et al. 2014). Recorded in Europe on the island of Lampedusa, Italy (Pagliano 2017) and from Malta (new record).

In addition to morphology, our species identification was also supported by molecular analyses. The BOLD Species Level Barcode Records Identification Engine placed our specimens within the BIN BOLD:ACO4425, which belongs to *M. aureola* with 99.7% to 100% matches to specimens collected from Saudi Arabia and Tunisia (Table 1; Fig. 5). Genetically, the specimens studied here were distinct from other species, including the closely related species *M. hyalina*, with a genetic match lower than 83% and *Campsomeriella thoracica* with a match lower than 80% (Table 1; Fig. 5). The genetic results confirmed the morphological identification of *M. aureola* from Malta.

Discussion

This study gives details of the first record of *M. aureola* from the Maltese Islands through the collection of two female specimens from Gozo. The species identification was supported by both morphological and molecular approaches, with the latter being added to the analyses as Osten (2000) indicated that it might be challenging to discriminate between male *M. aureola* and male *M. hyalina* morphologically. Therefore, even though we only examined female individuals in our study, genetic identification confirmed the species identity and increased

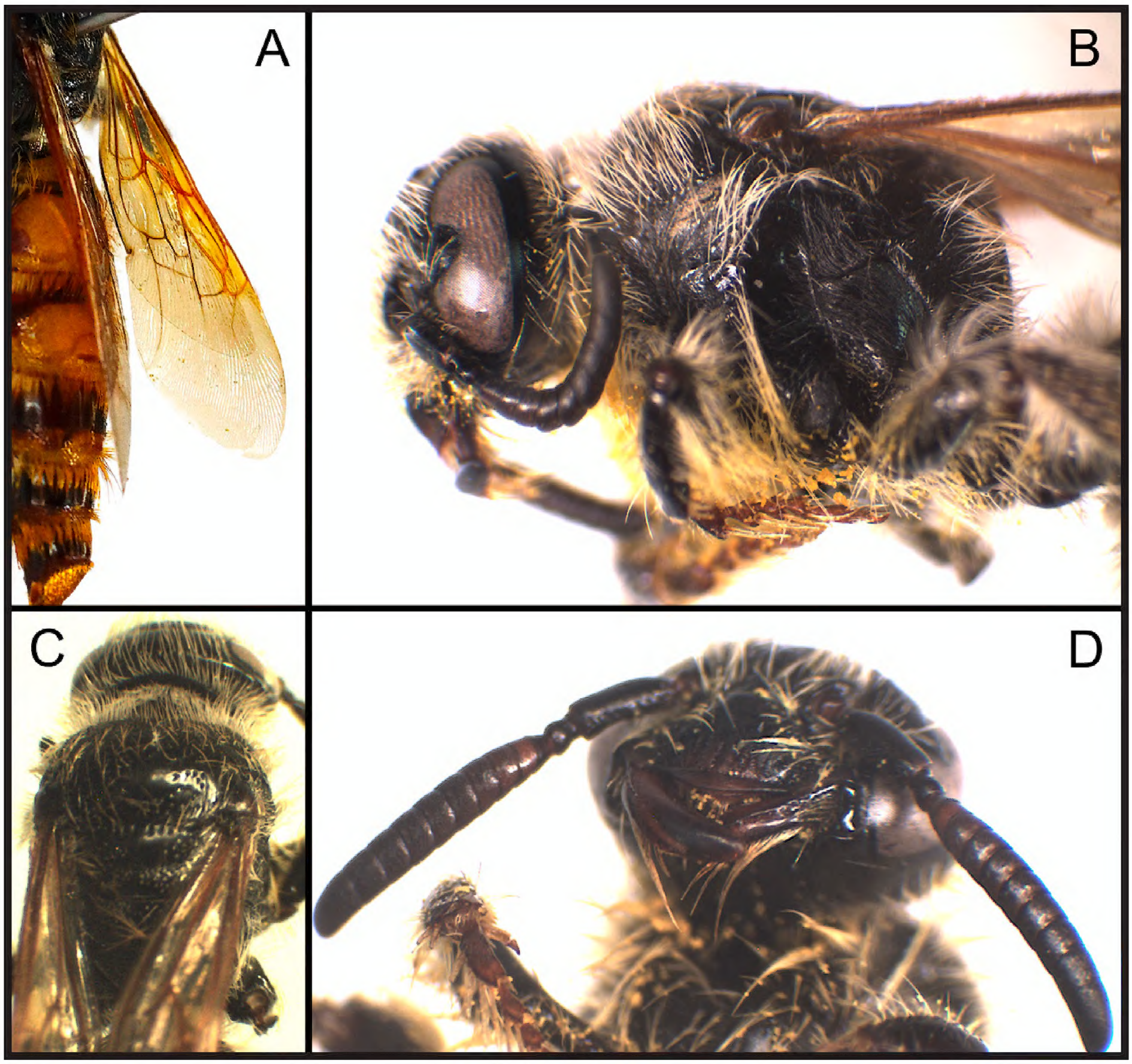

Figure 3. *Micromeriella aureola*. **A.** Wing pattern. **B.** Head area and thorax. **C.** Occupit and mesoscutum. **D.** Longitudinally ridged clypeus.

Table 1. The mean percentage genetic similarity within (diagonal in bold) and between (below diagonal) the various BOLD BINs representing specimens of *Micromeriella aureola*, *Micromeriella hyalina* and *Campsomeriella thoracica* (details of the data used is enlisted within Fig. 5).

Species		*M. aureola*	*M. hyalina*				*C. thoracica*		
BOLD BINs sample size		**ACO4425 (*n* = 7)**	**ACO5284 (*n* = 2)**	**ACO5577 (*n* = 1)**	**ACO5576 (*n* = 2)**	**ADX9764 (*n* = 3)**	**ADY3101 (*n* = 2)**	**ADY5639 (*n* = 4)**	**ADY5638 (*n* = 4)**
M. aureola	ACO4425	99.84							
M. hyalina	ACO5284	80.48	99.81						
	ACO5577	80.09	97.12	—					
	ACO5576	82.07	92.32	91.55	100.00				
	ADX9764	82.26	93.09	92.32	96.93	100.00			
C. thoracica	ADY3101	78.34	79.27	78.60	80.71	80.90	98.65		
	ADY5639	78.97	79.75	79.75	82.15	81.57	95.87	99.81	
	ADY5638	79.40	79.80	79.51	82.20	82.01	96.26	97.89	99.61

specific genetic data for *M. aureola* that would be required in the eventuality of encountering male specimens during biomonitoring surveys.

Recently this species has been recorded on the neighboring island of Lampedusa, Italy (Pagliano, 2017), 155 km south-west of the Maltese archipelago through two specimens identified as *M. aureola* possibly representing *M. aureola aureola* based on images in Pagliano (2017) following Osten (2000). Consequently, the current Maltese record represents the second European record for *M. aureola*, indicating a northwards expansion in this species' geographical distribution, possibly

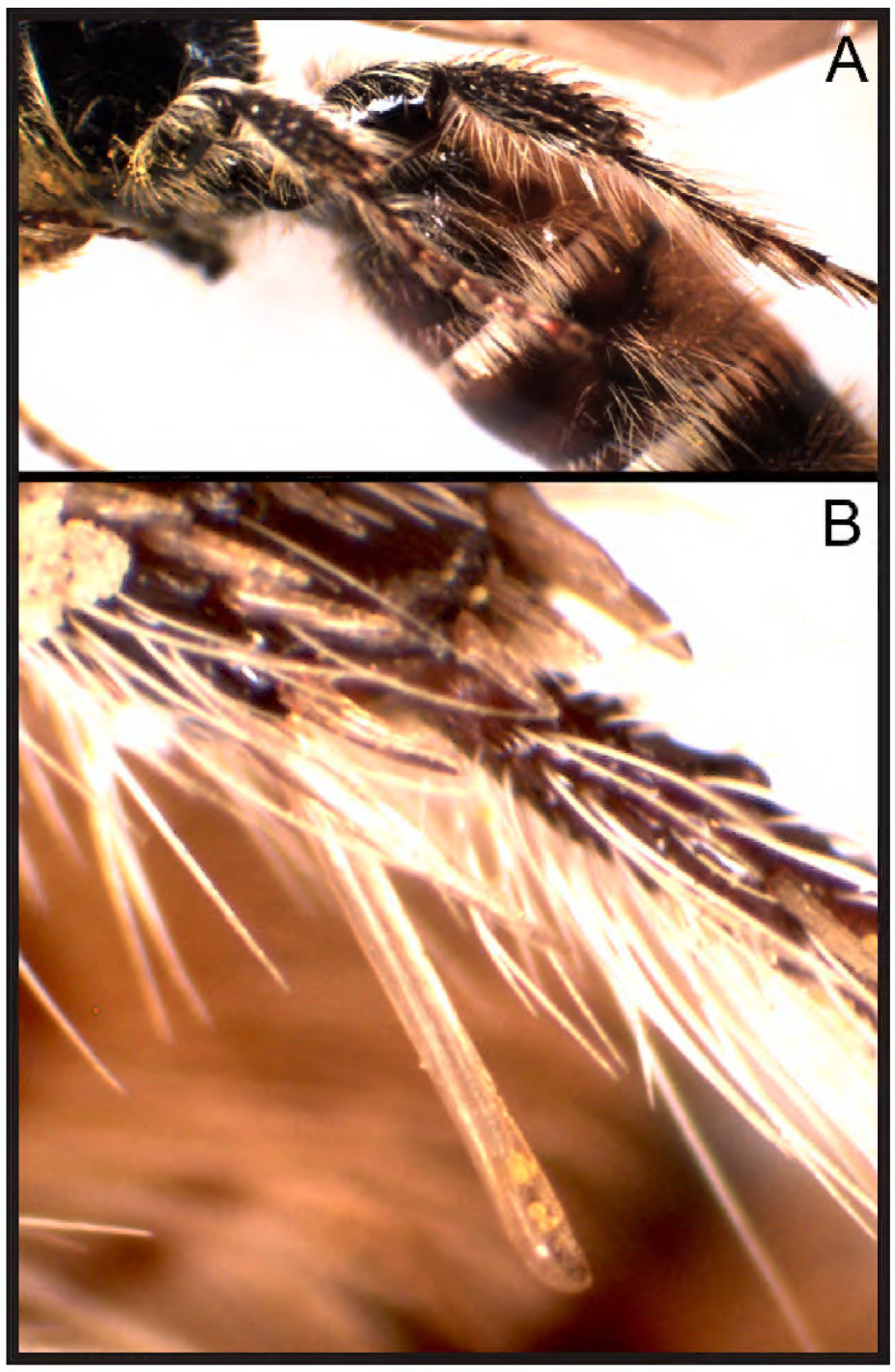

Figure 4. *Micromeriella aureola*. **A.** Abdomen area. **B.** Spatulate hind tibia.

associated to increasingly favorable environmental conditions as a result of climate change. The native range of *M. aureola* coincides with a warm dry climate (Osten 2000; Schmid-Egger 2017), which is also found in the Maltese Islands where the two recorded specimens were found. The sampling area was composed of an arid garrigue habitat towards the end of the dry season for the Maltese Islands. Given that the larvae of this species, like other Scoliidae, are parasitoids of beetles then its occurrence may be a threat to local endemic coleopteran fauna.

This study adds another species to the list of scoliid wasps for the Maltese Islands. It also highlights the importance of ongoing biodiversity fieldwork and DNA barcoding to fill knowledge gaps and aid conservation.

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Authors' Contributions

Conceptualization: AV. Data curation: AV. Formal analysis: AV, ST, NV. Funding acquisition: AV. Investigation: AV, NV. Methodology: AV, ST, NV. Project administration: AV. Resources: AV. Software: AV, NV. Supervision: AV. Validation: AV. Visualization: AV. Writing – original draft: AV, NV. Writing – review and editing: AV, NV.

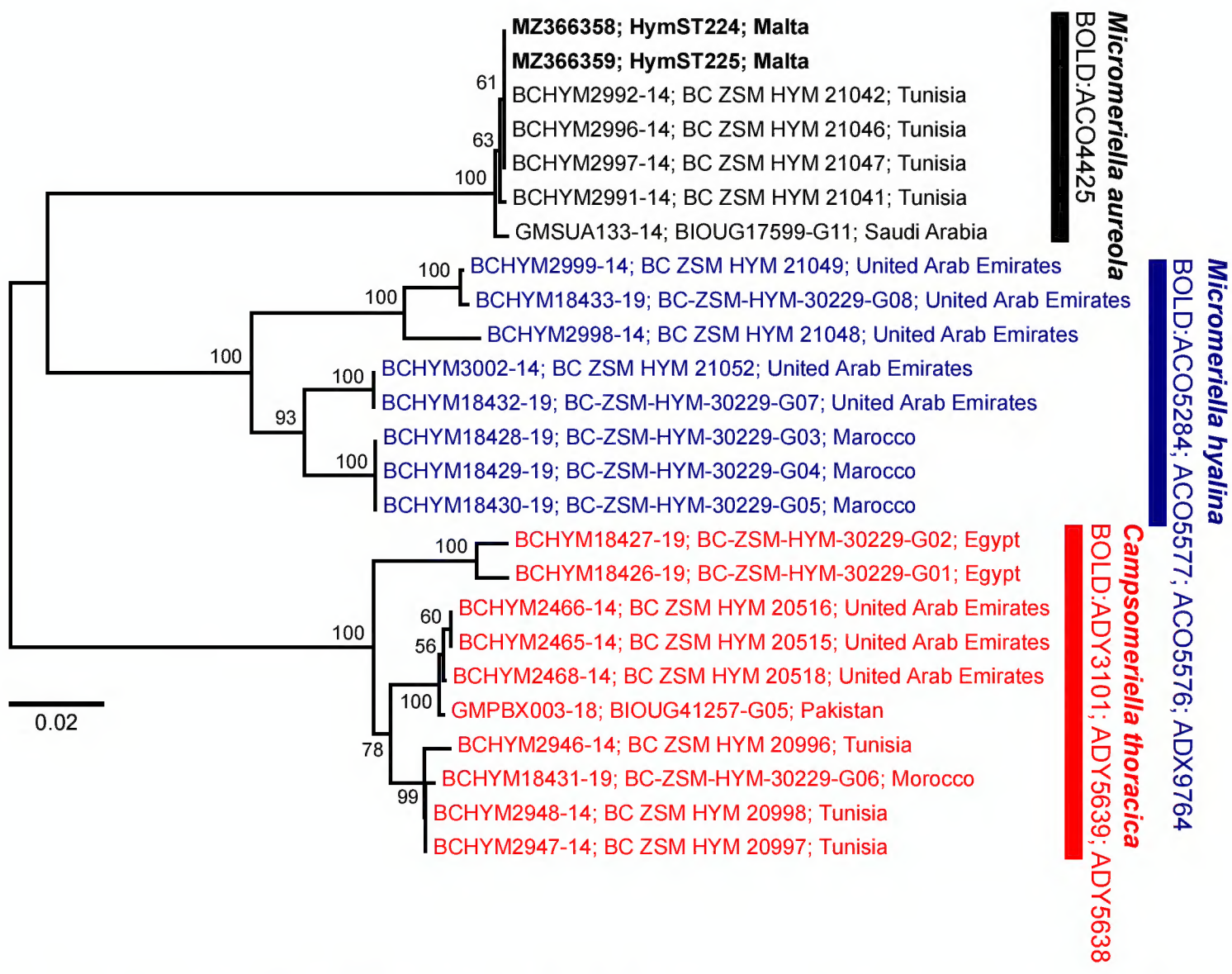

Figure 5. The phylogenetic relationship between the currently collected specimens and other *Micromeriella aureola* specimens (black), specimens of *Micromeriella hyalina* (blue) and *Campsomeriella thoracica* (red). Labels on the right include: BOLD or GenBank sequence code; specimen code; and country of collection. BOLD BINs are also included.

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